Zoosyst. Evol. 102 (1) 2026, 71–85 | DOI 10.3897/zse.102.161437

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Morphological and molecular data reveal one new species of the genus *Brachytarsophrys* Tian & Hu, 1983 (Anura, Megophryidae) from Guizhou, China

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Academic editor: Umilaela Arifin ♦ **Received** 7 June 2025 ♦ **Accepted** 21 November 2025 ♦ **Published** 5 January 2026

Abstract

We describe a new species of megophryid frog from Mt. Foding National Nature Reserve in Guizhou Province, China. The new species is morphologically distinguished from its congeners by a combination of the following characters: medium body size (SVL 88.1–93.2 mm in five adult males and 108.7–111.2 mm in two adult females); head wider than long; tympanum hidden; dorsal skin slightly rough with irregularly shaped dark blotches and stripes; chin, ventral surface of throat rough with brown, irregularly shaped dark markings, two large longitudinal black-brown stripes on lateral throat; ventral surface brown-black with small brown granular spots; occipital region slightly uplifted; upper eyelid with tubercles, one of which is prominent, bluntly conical horn; margin of tongue shallow notched behind; toes webbing formular: I (1½)-(2) II (1½)-(3) III (2½)-(4) IV (4)-(2) V, webbing extending as a wide fringe along either side of each toe; large single subgular vocal sac located laterally on floor of mouth in males; tadpoles with a transverse white stripe on ventral surface and two longitudinal white stripes along sides of the body. In addition, phylogenetic analysis of 16S rRNA and COI fragment confirms its distinct identity, which clusters the new species closest to *B. orientalis*, with uncorrected *p*-distances of COI at least 3.77%. At present, the new species is known only from small streams in Mt. Foding National Nature Reserve at about 740–1000 m elevation. Its natural history and conservation status is discussed.

Key Words

Brachytarsophrys, Guizhou Province, Mt. Foding National Nature Reserve, new species, phylogeny, taxonomy

Introduction

The Guizhou Karst Plateau, located at the uplifted edge of the Qinghai-Tibet Plateau, is the center of the Asian karst region. It exemplifies the typical inland karst plateau environment, characterized by underground drainage systems with sinkholes and caves (Zhu and Lin 1996; Ford and Williams 2007; Yue et al. 2018). Mt. Foding is located in the northeast of Guizhou, lying between Mt. Fanjing and Mt. Leigong. It can be regarded as a "bio-corridor" nature reserve, contributing to connectivity between the eastern and southeastern areas of Guizhou. Elevation within the reserve ranges from 500 to 1869 meters (Yu et al. 2016). The area represents a classic example of a montane hotspot for biodiversity and endemicity, although little is documented about its herpetofauna (Yu et al. 2016; Gu et al. 2018).

Genus *Brachytarsophrys* (family Megophryidae), occurs widely throughout the Indochina Peninsula and

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southern China (Frost 2025). These toad-like frogs inhabit montane streams surrounded by moist, subtropical, evergreen, broadleaf forests and exhibit cryptic behavior (Li et al. 2020). Although the higher-level generic taxonomy and phylogeny within the subfamily Megophryinae is still debated, the inner group of *Brachytarsophrys* can easily be distinguished from other members of Megophryinae. *Brachytarsophrys* is diagnosable based on several aspects, most notably by morphology, phylogeny, and ecology (e.g., Taylor 1962; Fei et al. 2009; Chen et al. 2017; Li et al. 2020). Currently, nine species have been described: *B. carinense*, *B. intermedia*, *B. orientalis*, *B. popei*, *B. chuannanensis*, *B. platyparietus*, *B. feae*, *B. qiannanensis* and *B. wenshanensis*. Two species, *B. carinense* and *B. intermedia*, occur in the Indochina Peninsula. The remaining seven, *B. orientalis*, *B. popei*, *B. chuannanensis*, *B. platyparietus*, *B. feae*, *B. qiannanensis* and B. *wenshanensis*, are distributed in Southern China (Li et al. 2020; Li et al. 2022; He et al. 2024; Amphibian China 2025). During herpetological surveys in Mt. Foding National Nature Reserve, Guizhou Province, China from 2019 to 2020, several specimens of *Brachytarsophrys* were collected. Subsequent study incorporating morphological and molecular data revealed that the sampled populations represent a distinct evolutionary lineage that warrants formal recognition as a distinct species.

Materials and methods

Sampling

A total of seven frogs and four tadpoles were collected during fieldwork from July 2019 to April 2020 in Mt. Foding National Nature Reserve, Guizhou Province (Fig. 1). After photographs were taken, the frogs were euthanized using benzocaine. Liver tissues were sampled and stored in 95% ethanol for DNA extraction. The specimens were fixed in 10% formalin and later transferred to 75% ethanol for long-term storage. These newly collected voucher specimens were deposited at the Museum of Biology of the Guizhou Institute of Biology (GIB), Guizhou Academy of Sciences, Guiyang, China. Research protocols were approved by the Ethics Committee of the Guizhou Institute of Biology, Guizhou Academy of Sciences.

DNA sequencing and analysis of sequences

Standard phenol-chloroform extraction with ethanol precipitation protocols were used to extract genomic DNA from collected tissues (Sambrook et al. 1989). Two mitochondrial gene segments were sequenced: 16S ribosomal RNA (16S rRNA) and cytochrome oxidase subunit I (COI).

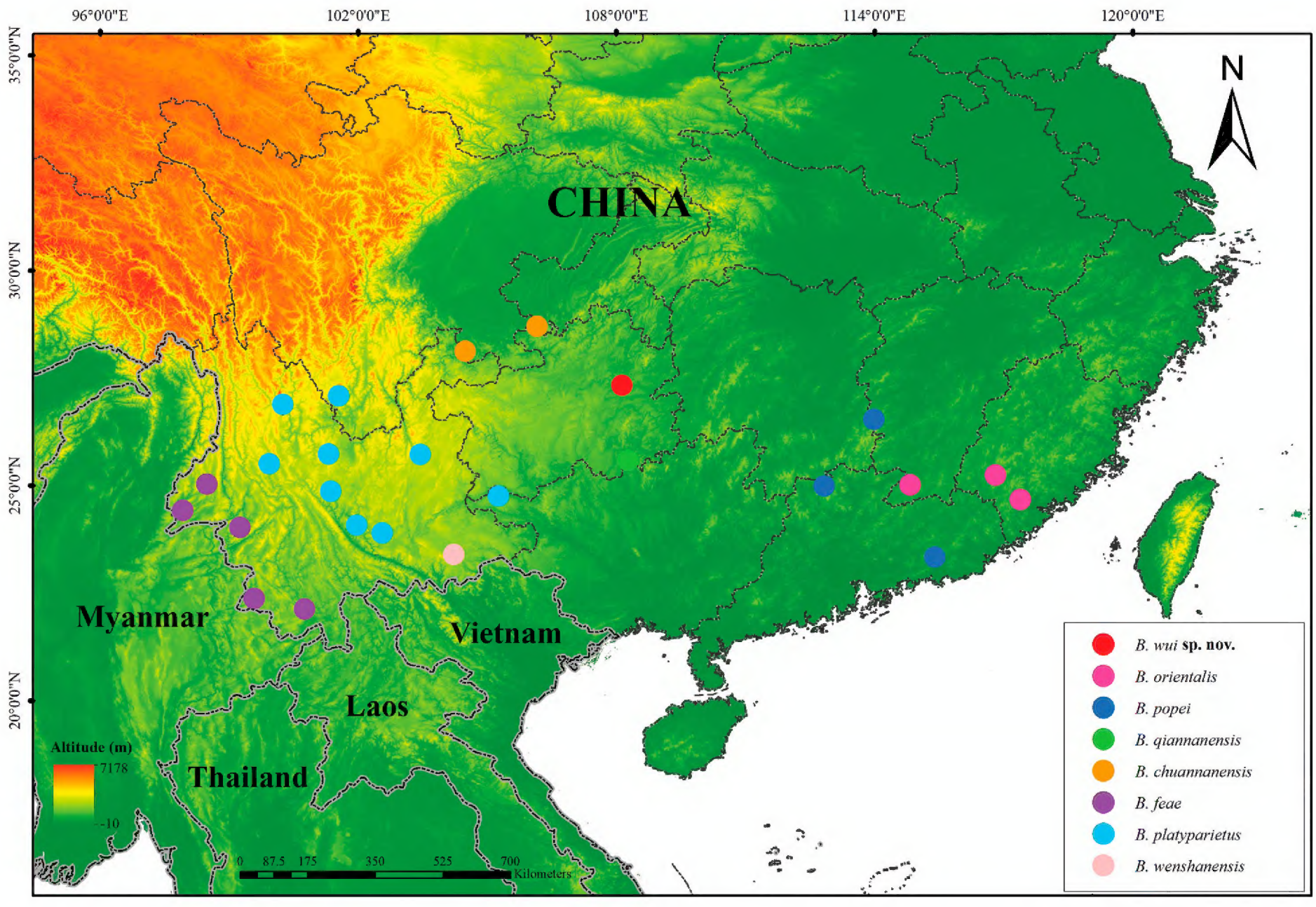

Figure 1. Collection locality (Mt. Foding, red dot) of *Brachytarsophrys wui* sp. nov. specimens from Mt. Foding, Guizhou used in this study and all the distribution of *Brachytarsophrys* in China.

Amplified PCR products were purified using enzymatic purification via exonuclease 1 and shrimp alkaline phosphatase that was incubated at 37 °C for 15 min followed by 80 °C to deactivate the enzymes. Purified PCR products were used as templates for Big Dye (Applied Biosystems, Foster City, CA) cycle sequencing. We conducted all sequencing on ABI 3100 automated sequencers (Applied Biosystems) at Suoqin, Beijing. All six new sequences were deposited in GenBank (see Table 1).

The newly obtained nucleotide sequences were first assembled and edited using DNASTAR LASERGENE 7.1. New sequences incorporated with homologous data retrieved from GenBank were aligned using MUSCLE 3.8 (Edgar 2004), then trimmed and inspected by eye for accuracy in MEGA 6 (Tamura et al. 2013). The phylogenetic reconstruction was carried out using maximum likelihood (ML) and Bayesian inference (BI). The GTR+G+I model was chosen as the best-fit model for 16S (Darriba et al. 2012).The BI analysis was performed on CIPRES Science Gateway (Miller et al. 2010) using MrBayes v3.2.4 (Ronquist et al. 2012). Four Markov chains were run for 10 million generations, with sampling every 1,000 generations. The first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to construct a consensus tree and estimate Bayesian posterior probabilities (BPPs). Convergence was assessed based on the average standard deviation of split frequencies (below 0.01) and effective sample size (ESS) values (greater than 200) in TRACER v1.5 (Rambaut and Drummond 2009). The ML analyses were performed using RAxML-HPC BlackBox 8.2.10 on the CIPRES Science Gateway (Miller et al. 2010). The analyses used the proportion of invariable sites estimated from the data and 1000 bootstrap pseudo replicates (random seed value: 12,345) under the GTR+I+G substitution model (Stamatakis 2014).Genetic divergences among *Brachytarsophrys* taxa were calculated using uncorrected p-distances base on 16S rRNA and COI, implemented in MEGA 6 with complete deletion of gaps and missing data (Tamura et al. 2013).

Advertisement calls analyses

Advertisement calls were recorded in the field using an ICD recorder (Sony ICD-TX50). Calls were recorded at a distance of about 1.5–2.0 m, and the ambient temperature was recorded using TP-2200 (A-volt). Call recordings were analyzed using Raven Pro 1.3 software (http://www.birds.cornell.edu/brpraven/RavenOverview.html), with the same protocol as reported in Köhler et al. (2017) and Rowley et al. (2017). Audio spectrograms for analysis were calculated with fast-Fourier transform (FFT) of 512 points, 50% overlap, and 172 Hz grid-spacing, using Hanning windows (Rowley et al. 2017). The call duration, intercall interval, number of notes per call, and dominant frequency were included in our analyses.

Morphology and morphometrics

For the specimens listed in Table 2, all linear measurements were obtained to the nearest 0.1 mm with digital calipers following Fei et al. 2009 and Watters et al. 2016, the seven adult individuals were measured for the following characters: snout-vent length (SVL): measured from tip of snout to vent; head length (HDL): measured from tip of snout to rear of jaw; maximum head width (HDW): measured width of head at its widest point; snout length (SNT): measured from tip of snout to anterior corner of ocular aperture; eye diameter (ED): diameter of exposed portion of eyeball; interorbital distance (IOD): measured at narrowest point between eyes on top of the head; internarial distance (IND): distance between nares; measured as maximal diameter of tympanum; width of upper eyelid (UEW): maximum width of upper eyelid; distance from nostril to eye (DNE): distance from the front of the eye to the center of the nostril; distance from nostril to snout (SN): distance from the center of the nostril to the tip of the snout; tibia length (TL): distance from knee to heel; hand length (HL): distance from the posterior end of the inner metacarpal tubercle to tip of third finger; hindlimb length (HLL): hindlimb length from tip of fourth toe to vent; foot length (FL): distance from the proximal end of inner metatarsal tubercle to the tip of fourth toe.

The toe webbing formula followed the protocol described by Savage (1975). Sex was determined by observation of secondary sexual characters, i.e., presence of internal vocal sac openings and nuptial spines in males. Tadpole developmental staging and external morphology were identified following Gosner (1960).

The tadpole was assigned to the new species based on its mitochondrial 16S rRNA and COI sequence identity (GIB20408, Genbank see Table 1), with the reference sequence obtained from examined adult specimens of the new species. Morphometrics of a single tadpole followed Ohler et al. (2011), and measurements of tadpole included maximum body height (BH); body length (BL); maximum body width (BW); maximum eye diameter (ED); maximum tail height (MTH): distance from the tip of the snout to the opening of the spiracle (SS); maximum tail muscle width (TMW): tail length (TAL); and total length (TOL).

Results

Phylogenetics and genetic divergence

A total of 403 base pairs (bp) of the 16S rRNA gene and 554 bp of the COI gene were concatenated, the alignment contained 238 variable positions and 169 parsimony-informative sites. The BI and ML analyses resulted in essentially identical topologies (Fig. 2). The 16S rRNA and COI matrilineal genealogy of *Brachytarsophrys* generally agreed with the phylogenetic relationships reported by He et al. (2024). *Brachytarsophrys* was divided into two

Table 1. List of voucher specimens and GenBank accession numbers for all DNA sequences of the 16S rRNA and COI mt DNA included in this study.

ID	Species	Locality	Voucher NO.	16S	COI	References
1	*Brachytarsophrys wui* sp. nov.	China: Mt. Foding, Shiqian County, Guizhou	GIB1972725	PX635278	PX568569	This study
2	*Brachytarsophrys wui* sp. nov.	China: Mt. Foding, Shiqian County, Guizhou	GIB1972746	PX635279	PX568570	This study
3	*Brachytarsophrys wui* sp. nov.	China: Mt. Foding, Shiqian County, Guizhou	GIB20408	PX635277	PX568568	This study
4	*Brachytarsophrys carinense*	Thailand: Doi Chiang Dao, Chiang Mai	K3001	KR827713	KR087626	Grosjean et al. (2015)
5	*Brachytarsophrys intermedia*	Vietnam: Krong Pa, Gia Lai	ROM 23794	/	MT162643	Li et al. (2020)
6	*Brachytarsophrys orientalis*	China: Jiulianshan Nature Reserve, Longnan County, Jiangxi	SYS a004225	OQ180989	MT162625	Li et al. (2020); Lyu et al. (2023)
7	*Brachytarsophrys popei*	China: Taoyuandong Nature Reserve, Yanling County, Hunan	SYS a001864	KM504256	MH406361	Zhao et al. (2014); Liu et al. (2018)
8	*Brachytarsophrys popei*	China: Taoyuandong Nature Reserve, Yanling County, Hunan	SYS a001865	KM504257	MT162620	Zhao et al. (2014); Liu et al. (2018)
9	*Brachytarsophrys popei*	China: Taoyuandong Nature Reserve, Yanling County, Hunan	SYS a001866	KM504258	MT162621	Zhao et al. (2014); Liu et al. (2018)
10	*Brachytarsophrys chuannanensis*	China: Zihuai Township, Hejiang County, Sichuan	SYS a004926	MH406901	MH406364	Liu et al. (2018)
11	*Brachytarsophrys chuannanensis*	China: Zihuai Township, Hejiang County, Sichuan	SYS a004927	MH406902	MH406365	Liu et al. (2018)
12	*Brachytarsophrys platyparietus*	China: Duodihe, Dayao county, Yunnan	SYS a005919	OQ180990	MT162633	Li et al. (2020); Lyu et al. (2023)
13	*Brachytarsophrys platyparietus*	China: Malipo County, Yunnan	Yuan10871	PP411773	PP235298	Wu et al. (2024)
14	*Brachytarsophrys feae*	China: Huangcaoling, Yunnan	KIZ046706	KX811810	KX812056	Chen et al. (2017)
15	*Brachytarsophrys feae*	China: Jingdong County, Yunnan	SYS a003912	MH406899	MH406362	Liu et al. (2018)
16	*Brachytarsophrys feae*	China: Jingdong County, Yunnan	SYS a003913	MH406900	MH406363	Liu et al. (2018)
17	*Brachytarsophrys feae*	China: Longchuan, Yunnan	KIZYN070570	KX811809	KX812055	Chen et al. (2016)
18	*Brachytarsophrys qiannanensis*	China: Libo County, Guizhou	CIBLB20210806053	OK104099	OK104052	Li et al. (2022)
19	*Brachytarsophrys qiannanensis*	China: Libo County, Guizhou	CIBLB20210806054	OK104100	OK104053	Li et al. (2022)
20	*Brachytarsophrys qiannanensis*	China: Libo County, Guizhou	CIBLB20210806055	OK104101	OK104054	Li et al. (2022)
21	*Brachytarsophrys qiannanensis*	China: Libo County, Guizhou	CIBLB20210806056	OK104102	OK104055	Li et al. (2022)
22	*Brachytarsophrys wenshanensis*	China: Xichou, Yunnan	SWU 0004072	PP153337	PP155195	He et al. (2024)
23	*Brachytarsophrys wenshanensis*	China: Xichou, Yunnan	SWU 0002976	PP153338	PP155198	He et al. (2024)
24	*Brachytarsophrys wenshanensis*	China: Xichou, Yunnan	Yuan 25290	PP153339	PP155197	He et al. (2024)
25	*Brachytarsophrys wenshanensis*	China: Xichou, Yunnan	Yuan 25291	PP153336	PP155196	He et al. (2024)
26	*Atympanophrys shapingensis*	/	CIB SC2011102004	JX458090	JX458090	Xiang et al. (2012)

"/" means this character is unknown.

Table 2. Morphometric measurements (in mm) of *Brachytarsophrys wui* sp. nov. See methods section for abbreviation definitions.

Voucher number	GIB1972746	GIB1972747	GIB1972748	GIB1972749	GIB1972750	GIB1972751	GIB1972725
Sex	**Male**	**Male**	**Male**	**Male**	**Male**	**Female**	**Female**
SVL	90.8	93.2	88.6	88.1	93.1	111.2	108.7
HDL	32.9	33.4	28.5	28.2	33.6	38.8	38.3
HDW	45.7	48.1	43.2	44.4	47.2	56.7	57.4
SNT	6.4	5.5	5.6	4.9	5.9	7.1	6.9
IND	8.2	9.2	7.8	8.3	8.3	10.5	10.6
IOD	9.4	11.9	9.8	10.9	11.9	13.4	12.5
UEW	7.7	7.8	7.8	7.4	7.9	7.5	7.8
ED	9.1	8.7	9.4	7.4	8.2	9.5	10.5
DNE	3.5	3.4	3.4	3.4	3.7	5.6	5.1
SN	2.4	1.9	2.1	1.3	2.1	1.8	1.9
HL	42.5	42.1	40.5	40.3	42.3	49.6	49.8
HLL	124.7	131.6	118.7	122.1	127.4	154.2	151.4
TL	37.8	40.2	37.3	38.3	41.2	46.7	44.1
FL	39.3	43.3	38.2	37.7	38.4	52.6	49.6

Holotype: GIB1972747

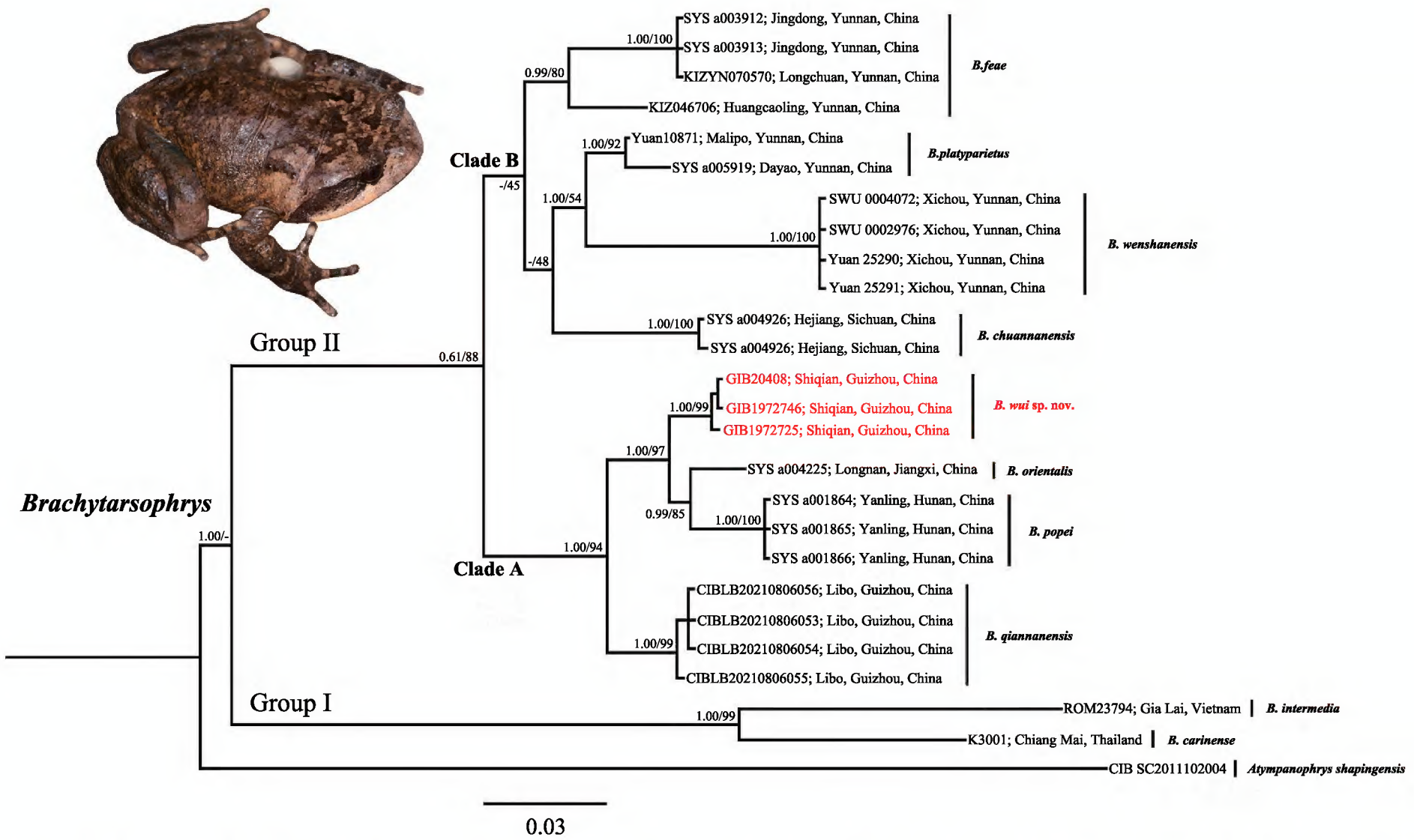

Figure 2. Bayesian phylogram of *Brachytarsophrys* inferred from partial DNA sequences of the 16S rRNA and COI gene. Node support is indicated on branches as Bayesian posterior probabilities (displayed values > 0.95 values) and maximum likelihood bootstrap support displayed values > 70 values). The scale bar represents 0.03 nucleotide substitutions per site.

highly divergent groups with strong support (Group I and II; Fig. 2). Group I contains two species (*B. carinense* and *B. intermedia*) from the Indochina Peninsula. All Chinese species belong to Group II, which itself contains two well-supported clades (Clades A and B; Fig. 2). Clade B includes four known species mostly from southwestern China (*B. platyparietus*, *B. chuannanensis*, *B. wenshanensis* and *B. feae*). Clade A contains samples mostly from southeastern China, including *B. qiannanensis*, *B. popei*, *B. orientalis*, and those from Mt. Foding, Guizhou Province, China. Mt. Foding samples formed a strongly independent lineage (BPP = 1.00, BS = 99; Fig. 2) and were genetically identical to each other.

The genetic distances between this novel lineage and other *Brachytarsophrys* species ranged from 0.00% to 5.96% in 16S rRNA sequences (Suppl. material 1), from

2.83% to 16.98% in COI sequences (Suppl. material 2). This lineage was the sister taxon to a monophyletic group composed of *B. Popei and B. orientalis* with moderate genetic difference (mean *p*-distance 3.77% in COI; Li et al. 2020). This indicates that the individuals found at Mt. Foding are a distinct, undescribed evolutionary lineage. Based on the results of the morphological examination presented below and phylogenetic analysis, the specimens from Mt. Foding Nature Reserve are regarded as a new species described herein.

Taxonomy

Our results indicated that the observed molecular divergence of the newly documented population of *Brachytarsophrys* from Mt. Foding, Guizhou is concordant with stable differences in diagnostic morphological characters, which distinguish it from all known congeners. Thus, upon combining morphological and molecular evidence, we herein describe this distinct lineage of *Brachytarsophrys* as a new species.

Brachytarsophrys wui Lyu, Zhou & Long sp. nov.

https://zoobank.org/D63F61AE-C8F3-4599-97BF-5DB5B49D5CD7

Type material. ***Holotype*** • GIB1972747, adult male, from Lengshui River of Mt. Foding Nature Reserve, Shiqian County, Guizhou Province, China (27.326006°N, 108.142440°E, elevation 740–1000 m. a.s.l.; Fig. 1), collected by Jingcai Lyu on 28 July 2019.

Paratypes • Six specimens (two adult females, four adult males): GIB1972746, GIB1972748–50, adult males, from the type locality collected by Jingcai Lyu on 28 July 2019 • GIB1972725, adult female, collected by Shenglun Zhou on 27 July 2019, from Binjia River • GIB1972751, adult female, collected by Shenglun Zhou on 23 July 2020 from Lengshui River of Mt. Foding.

Reference specimens. GIB1972709, GIB20408, two tadpoles were collected by Jingcai Lyu on 29 July 2019 and 8 April 2020 from Lengshui River of Mt. Foding.

Diagnosis. *Brachytarsophrys wui* sp. nov. can be distinguished from its congeners by the following combination of morphological characters: (1) medium body size (SVL 88.1–93.2 mm in five adult males and 108.7–111.2 mm in two adult females); (2) head very large, wider than long, and nearly one-half as broad as the SVL; (3) tympanum hidden; (4) pupils black, iris brownish red; (5) upper eyelid with tubercles, one of which is enlarged and becoming a prominent, bluntly conical horn; (6) dorsal skin slightly rough with irregularly shaped dark blotches and stripes; (7) belly, chest, and limbs smooth with irregular off-white spots; (8) chin, ventral surface of throat rough with dark brown, irregularly shaped markings, two large longitudinal black-brown stripes on lateral throat; (9) single subgular vocal sac openings slit-like, located posterolateral on floor of mouth in male; (10) indistinct brown nuptial pad on the dorsal surface of the first and second fingers base, with dense, tiny, black nuptial spines in adult male during breeding season; (11) forelimb short and thick, webbing absent, One third as broad as distal toe phalanx; (12) relative length of fingers I<II<IV<III; (13) toes about I (1½)-(2) II (1½)-(3) III (2½)-(4) IV (4)-(2) V webbed in male; (14) tadpoles with a transverse white stripe, white speckles on posterior ventral surface, and two longitudinal white stripes along the sides of body; (15) oral disk with lips expanded vertically forming dorsally oriented funnel.

Description of the holotype. GIB1972747, Adult male, SVL 93.2 mm; head very large and extremely depressed, wider than long (HDW/HDL = 1.5), and nearly one-half as broad as the SVL (Fig. 3A); choanae large, ovoid, close to the tip of snout, partly concealed by the maxillary shelves; internasal region slightly concave; snout tip in both ventral view and lateral view protruding slightly beyond lower jaw; interorbital region flatted; interorbital distances lightly wider than upper eyelid, 1.14 times as broad as internasal distance; pupil vertical; tympanum completely hidden (Fig. 4B); canthus rostral is distinct; eyes large (EYE/HDL = 0.27), eye diameter slightly longer than snout length (EYE/SNT = 1.12); two vomerine ridges markedly elevated, extending behind far beyond the posterior of the choanae; vocal sac openings slit-like, located posterolateral on floor of mouth; tongue pyriform, with a shallow notch at posterior tip; supratympanic ridge distinct, running from eye towards axillary region with raised tubercles; occipital region slightly uplifted; a distinct transverse groove posteriorly demarks the head (Fig. 4A). Forelimbs short and thick, fingers long and quite fat, with absent webbing and narrow fringes; relative length of fingers I<II<IV<III (Fig. 4F); tips of fingers rounded and slightly swollen; two metacarpal tubercles present, inner one significantly enlarged, outer one slightly enlarged (Fig. 4G); hindlimbs relatively long and robust, tibiotarsal articulation reaching to the commissure of the jaw when leg is stretched along the side of the body; the heels separate when legs are held at right angles to the body axis; tibia two-fifths snout-vent length (TIB/SVL = 0.42); relative toe length I<II<V<III<IV; toe tips rounded and thickened; fringes on toes slight smaller; toes webbing markedly developed about I (1½)-(2) II (1½)-(3) III (2½)-(4) IV (4)-(2) V webbed, digits without subarticular tubercle, inner metatarsal tubercle ovoid and as long as the first toe, outer metatarsal tubercle and tarsal fold absent (Fig. 6A2). Dorsal skin slightly rough with several conical tubercles, irregular pustules on flanks and dorsum of body and limbs (Fig. 4E); chin, ventral surface of throat trough with brown, irregular dark markings, two large longitudinal black-brown stripes on lateral throat (Fig. 4D); supratympanic fold narrow and distinct, curving ventrally from posterior corner of eye to above insertion of arm; upper eyelid with tubercles, one of which is enlarged into a prominent, bluntly conical horn (Fig. 4C); belly, chest, and limbs smooth with irregular off-white spots; pectoral glands distinct, close to bases of forelimbs on ventral surface of axillary region; pectoral glands distinct (Fig. 4H); tiny nuptial spines densely covering the

Figure 3. *Brachytarsophrys wui* sp. nov. in life. **A.** Dorsolateral view of the adult male holotype GIB1972747; **B.** Dorsolateral view of the adult female paratype GIB1972725; **C.** Ventral view of the holotype GIB1972747; **D.** Ventral view of the paratype GIB1972725. Photos by Jing-Cai Lyu.

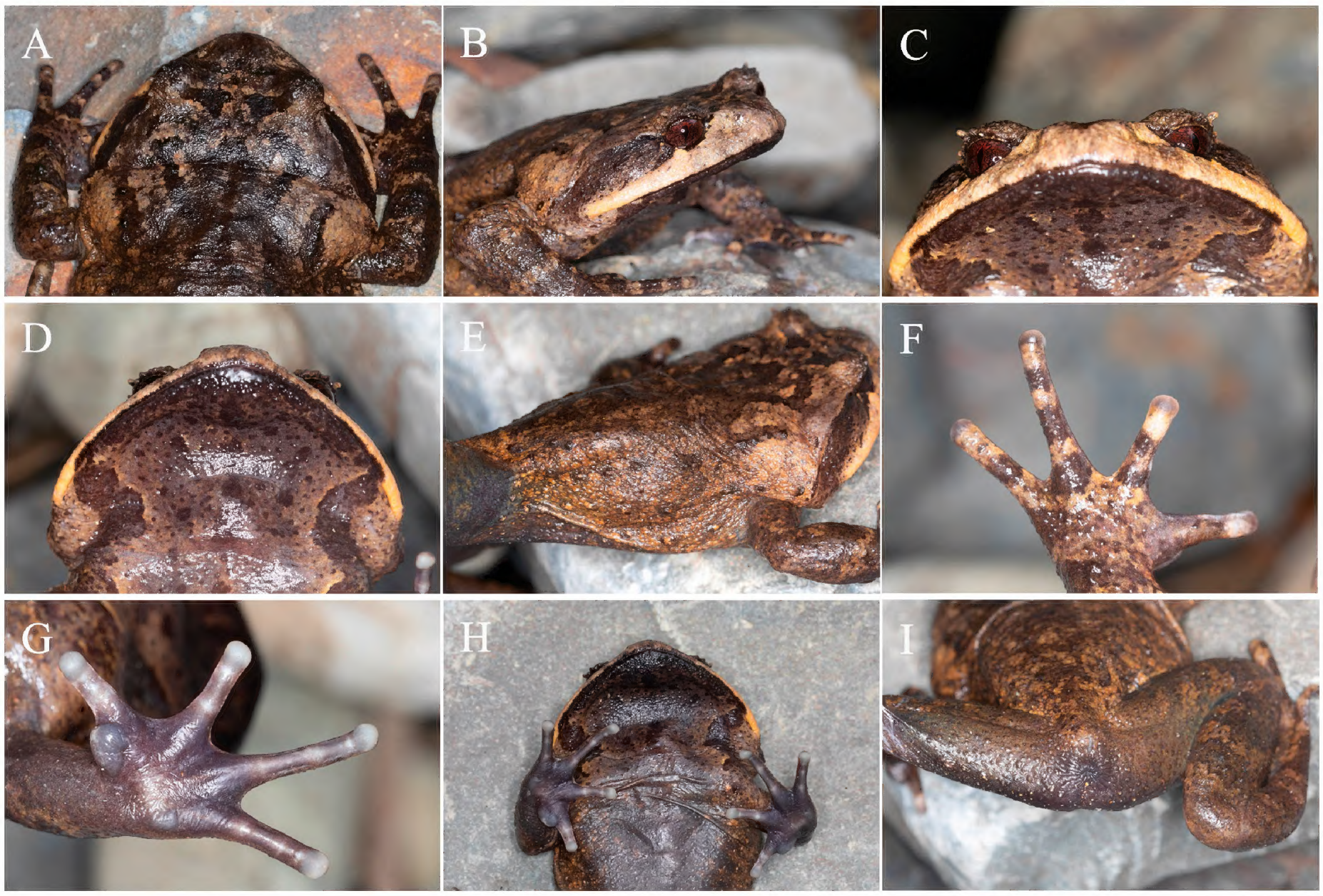

Figure 4. Holotype of *Brachytarsophrys wui* sp. nov. GIB1972747. in life. **A.** Dorsal view; **B.** Tympanum view; **C.** Upper eyelid with tubercles; **D.** Throat view; **E.** Lateral view; **F.** Left hand in dorsal view; **G.** Hand in ventral view; **H.** Pectoral gland area view; **I.** Cloacal area view. Photos by Jing-Cai Lyu.

dorsal surface of the first finger; cloacal opening unmodified, directed posteriorly at upper level of thighs (Fig. 4I).

Coloration of holotype. In life, dorsum brown, tinged with dark blotches and streaks; dark blotches towards the anterior of the dorsum increasingly edged with light brown, forming an irregular band of light brown between the eyes and a distinct W-shaped marking between the shoulders; tubercles black on rear of body, light brown or brown tinged with black on sides of body; ventral surface brown-black with small brown granular spots (Fig. 3C); pectoral glands yellowish.

Ventral surface of throat with brown, irregular dark markings, two large longitudinal black-brown stripes on lateral throat; the tubercles on upper eyelid light colored, horn yellowish; pupils black; iris brown, with black reticulations throughout; upper lip light brown; tympanic region brown, mottled with dark brown markings; transverse dark brown bars on dorsal surface of fingers, toes, lower arms, tarsus, thighs and tibia; femoral gland yellowish; nuptial spines black; lower surface of digits, webs, palms, two metacarpal tubercle, and soles pale blue-grey; tip of digits and inner metatarsal tubercle grey-white; belly, chest, and limbs with irregular off-white spots, several yellowish spots on two sides of belly.

In preservation: Color of dorsal surface fades to brownness; the streaks, interocular stripe on the dorsum and W-shaped ridges on shoulders fades indistinct (Fig. 5A); the throat and anterior chest fade to light brownish, while the abdomen grayish-white (Fig. 5B); tip of digits, fingers and toes grayish-white (Fig. 5C, D).

Tadpoles. GIB20408, in stage 29. BH 5.0 mm; BL 12.0 mm; BW 5.6 mm; ED 2.5 mm; MTH 6.4 mm; TMW 3.0 mm; TAL 24.3 mm; TOL 36.0 mm. TAL/TOL = 0.68. In life, body slender, flattened above (Fig. 7A1); tail depth almost equal to body depth; dorsal surface of head brown with irregularly shaped dark blotches and stripes; presence of unique color patterns on ventral surface (Fig. 7A3); two short, longitudinal white stripes bordered with blackish-brown on sides of ventral surface of head; a transverse white ventral stripe bordered with brown spots in line with the spiracles; two longitudinal white stripes along the sides of body, which begins at the end-points of the white transverse stripe and extend backwards to terminate near the anal tube (Fig. 7A2); belly mottled with dense white speckles between two longitudinal stripes; tail with three dark longitudinal stripes per side, one at base of the dorsal fin, one at base of the ventral fin, one centered along the caudal muscle.

Male secondary sexual characteristics. The presence of large single subgula vocal sac; small femoral gland on posterior of thigh; indistinct brown nuptial pad on the dorsal surface of the first and second fingers base, with dense, tiny black nuptial spines in all adult male specimens in preservative (Fig. 5E, F).

Morphological variation. Variation in size and body proportions of the type series are shown in Table 3. All four-adult male paratypes are very similar to the holotype in morphology and color pattern. In life, the female paratype GIB1972725 differs from males in the following: light brown dorsum, streaks on dorsum, dorsal surface of

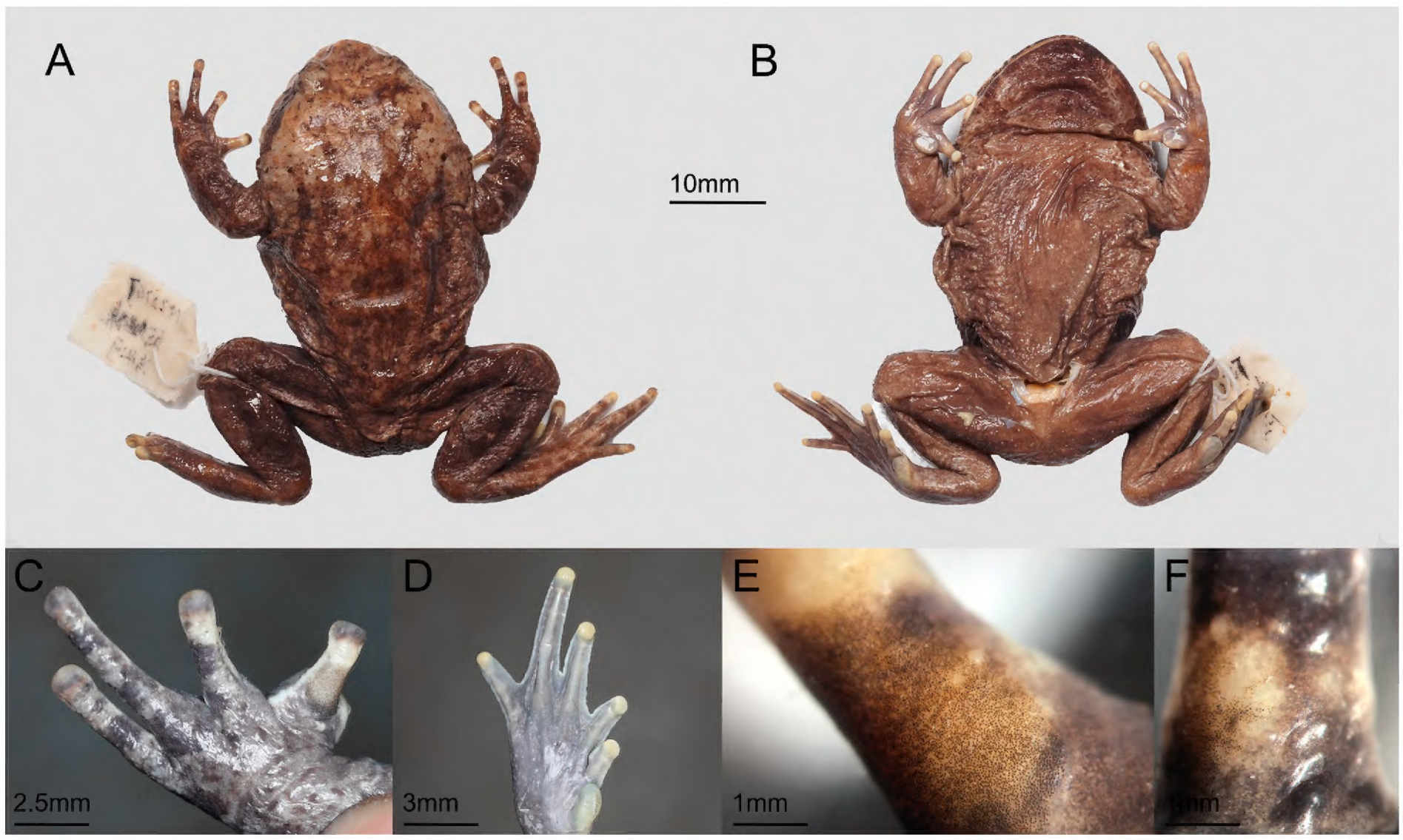

Figure 5. The holotype of *Brachytarsophrys wui* sp. nov. (GIB1972747, male) in preservative. **A.** Dorsal view; **B.** Ventral view; **C.** Dorsal view of hand; **D.** Ventral view of foot; **E.** Nuptial spines of the first finger base; **F.** Nuptial spines of the second finger base. Photos by Jin-Ming Chen (**A, B**) and Jing-Cai Lyu (**C–F**).

fingers, toes, lower arms, tarsus, thighs, and tibia indistinct (Fig. 3B, D); upper lip swollen and lighter colored; webs and fringes on toes slight smaller (Fig. 6A1); occipital region slightly uplifted. The color patterns on the ventral surfaces of 38th stage tadpoles are indistinct compared to 29th stage tadpoles, instead being oyster- white with sparse black speckles (Fig. 7B1, B2).

Etymology. The specific epithet "*wui*" is in honor of late professor and zoologist Lyu Wu (1915–1983), vice president (1969–1975) of Guizhou Academy of Sciences (Guizhou Province, China), in recognition of his efforts on biodiversity surveys and research in Guizhou Province in the 1960s and 70s. For the common name, we recommend "Wu's Short-Legged Toad" (English) and "Wu Shi Duan Tui Chan" (伍氏短腿蟾) (Chinese).

Natural history. All specimens were collected at night in small streams in Mt. Foding Nature Reserve at about 740–1000 m elevation. Calling males were found along the stream, perching under rocks (Fig. 8). Duck-like calls could be heard during July (Fig. 10). These frogs inhabit montane streams surrounded by moist, subtropical, evergreen, broadleaf forests. An adult male and a cluster of eggs (approximately 1,600 in total) were found under a rock, suggesting that adult males may exhibit egg protection behaviors (Fig. 9). We conducted investigations once a month from March to October and found the breeding season of this species is likely from July to August. Females collected during these months were gravid, and males were heard calling only from July to the beginning of August. During the surveys, the number of males observed was much greater than females (males: females = 16:2).

Advertisement calls. Call descriptions are on the basis of the calls of the holotype, recorded at an ambient temperature of 19.5 °C (Fig. 10C). Calls of *Brachytarsophrys wui* sp. nov. consisted of a duck-like single note, with call duration 0.104–0.124 s (0.111 ± 0.006, n = 21), call repetition rate 2.3 s and intercall interval 0.261–0.460 s (0.318 ± 0.055, n = 21). The dominant frequency was between 1.0–1.2 kHz, but fundamental frequency and harmonics were not clear (Fig. 10A, B).

Distribution. The species is currently only known from Mt. Foding National Nature Reserve, Shiqian County, Guizhou Province, China.

Comparisons. *Brachytarsophrys wui* sp. nov. differs from all other species of *Brachytarsophrys* by a combination of body size, ventral coloration and pattern, dorsal skin texture, degree of webbing and fringing on the fingers and toes, tadpole coloration and characteristics, and male secondary sexual characteristics (Kaewtongkum et al. 2014; Chuaynkern et al. 2022) (Table 3).

Care is taken here to specifically differentiate *Brachytarsophrys wui* sp. nov. from its closest congeners on the 16S rRNA and COI matrilineal genealogy, *B. orientalis* and *B. popei*. *Brachytarsophrys wui* sp. nov. differs from its sister taxon *B. orientalis* (Li et al. 2020) by the following: larger body size (SVL 108.7–111.2 mm in females, 88.1–93.2 mm in males vs. 88.6 mm in female, 76.8–82.7 mm in males); chin, ventral surface of throat rough (vs. smooth); the presence of a transverse white stripe on belly of tadpole (vs. absence); the presence of two large longitudinal black-brown stripes on lateral throat (vs. absence). Tadpole with a transverse white ventral stripe bordered with brown spots in line with the spiracles; two longitudinal white stripes along the sides of body, which begin at the end-points of the white transverse stripe and extend backwards to terminate near the anal tube (vs. absence of transversal stripes on chest). *Brachytarsophrys wui* sp. nov. can be differentiated from *B. popei* (Zhao et al. 2014) by having larger

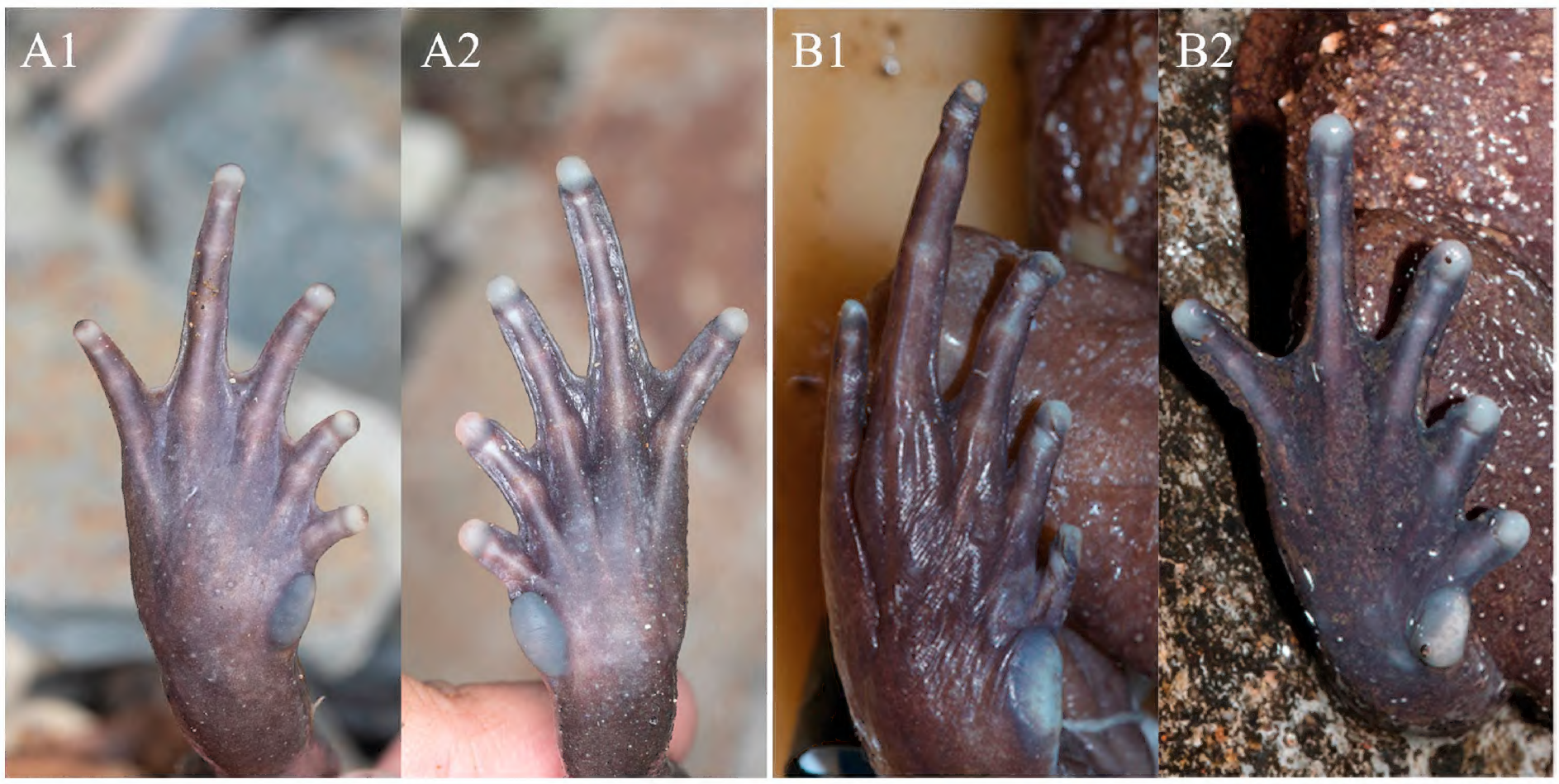

Figure 6. Morphological differences between *Brachytarsophrys wui* sp. nov. GIB1972725 and *B. orientalis*. **A1.** Ventral views of foot in female of *B. wui* sp. nov.; **A2.** Ventral views of foot in male of *B. wui* sp. nov.; **B1.** Ventral views of foot in female of *B. orientalis* (Li et al. 2020); **B2.** Ventral views of foot in male of *B. orientalis* (Li et al. 2020). Photos by Jing-Cai Lyu (**A1, A2**).

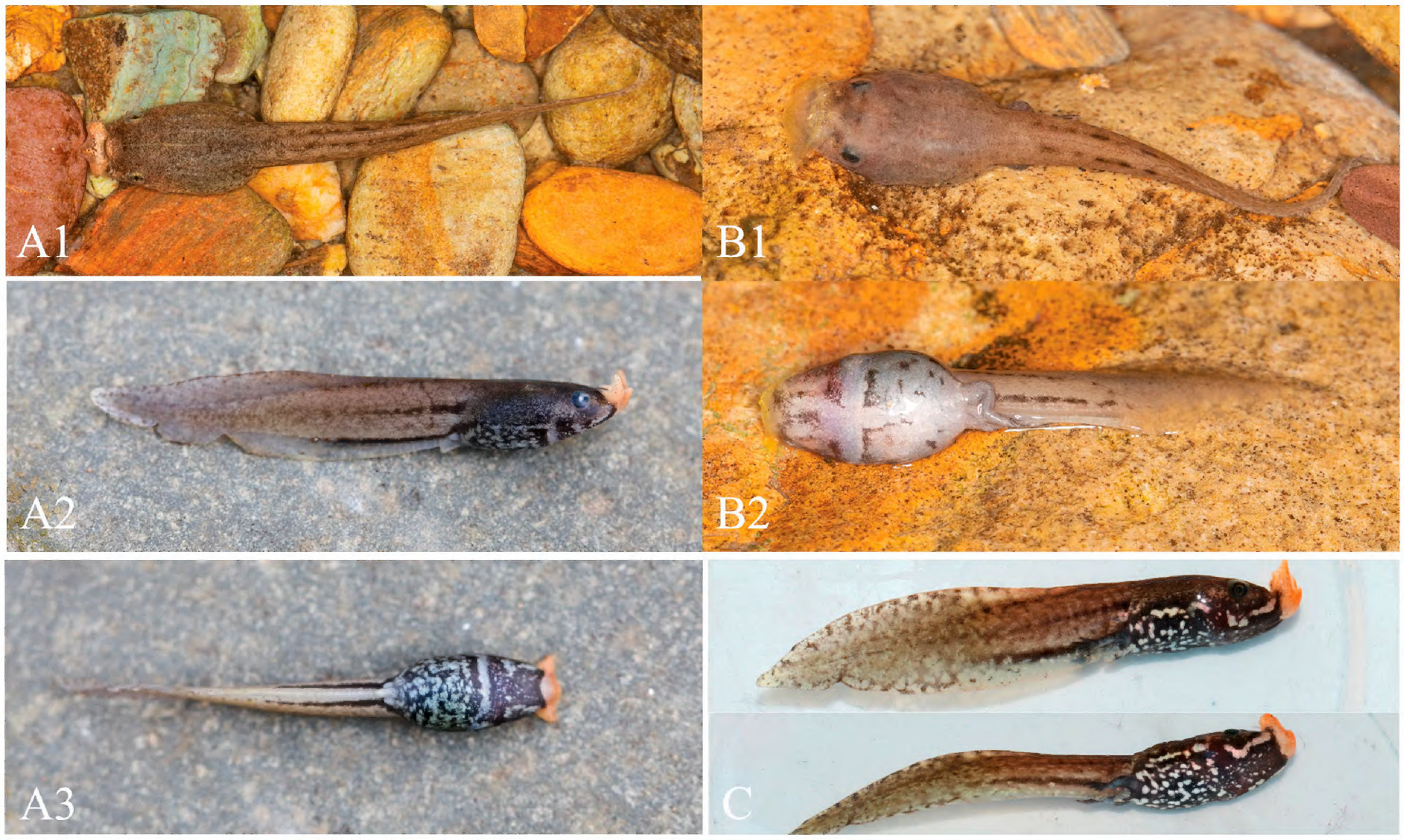

Figure 7. Morphological differences between *Brachytarsophrys wui* sp. nov. and *B. orientalis* of tadpoles. **A1–A3.** Dorsal, lateral, and ventral view of 29th stage tadpole of *B. wui* sp. nov. GIB20408; **B1, B2.** Dorsal and ventral view of 38th stage tadpole of *B. wui* sp. nov. GIB1972709; **C.** Lateral and ventral view of 36th stage tadpole of *B. orientalis* (Li et al. 2020). Photos by Jing-Cai Lyu (**A1–A3, B1, B2**).

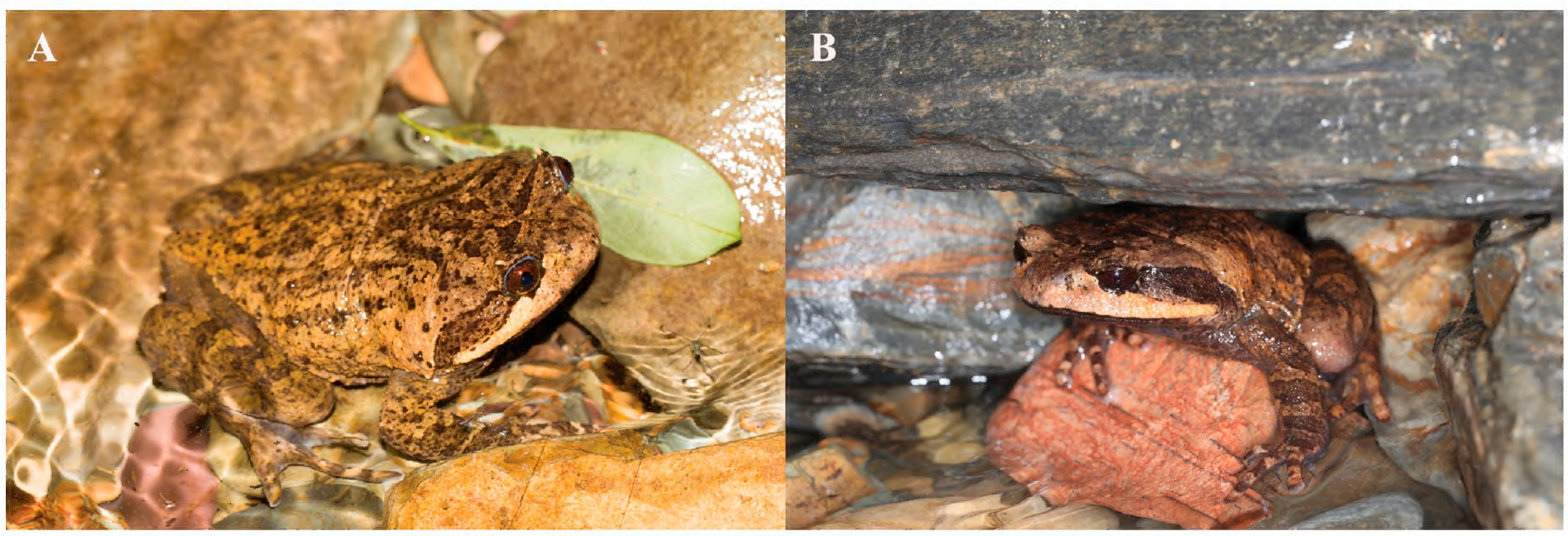

Figure 8. *Brachytarsophrys wui* sp. nov. GIB1972748 in habitat. Photos by Sheng-Lun Zhou (**A**) and Jing-Cai Lyu (**B**).

body size (vs. SVL 86.2 mm in female, 70.7–83.5 mm in males); tongue with feebly notched (vs. deep posterior notch), head width about 1.5 times head length (vs. head width about 1.2 times head length).

Brachytarsophrys wui sp. nov. can be further morphologically differentiated from the remaining *Brachytarsophrys* congeners. Differing from *B. carinense* (Boulenger, 1889), *Brachytarsophrys wui* sp. nov. has a smaller body size (vs. SVL124–168 mm in females, 91.6–123 mm in males), Differences of *Brachytarsophrys wui* sp. nov. from *B. intermedia* (Smith, 1921) having absence of a dermal ridge or glandular fold ondorsum (vs. presence of dermal ridge or glandular fold ondorsum). *Brachytarsophrys wui* sp. nov. differs from *B. chuannanensis* (Fei and Ye 2001) by having larger body size (vs. SVL92 mm in female, 91.4–109.4 mm in males); slightly larger webbing of relative length I (1½)-(2) II (1½)-(3) III (2½)-(4) IV(4)-(2) V in males, I (2)-(2^{+}) II (1⅔)-(3) III (3^{-})-(4) IV (4^{+})-(2½) V in females (vs. toes with rudimentary webbing, narrow, less than one fifth of distal toe phalanx in males). *Brachytarsophrys wui* sp. nov. differs from *B. platyparietus* (Li et al. 2020) by having lateral fringes on the toes of males one third as broad as distal toe phalanx (vs. half as broad as distal toe phalanx); head width about 1.5 times head length (vs. head width about 1.2 times head length), and the absence of small tubercles on pectoral region, lateral belly to lower flank of body, ventral surface,

Table 3. Diagnostic characters distinguishing the ten *Brachytarsophrys* species from each other.

Morphological characters	*B. wui* sp. nov.	*B. orientalis*	*B. popei*	*B. qiannanensis*	*B. carinense*	*B. platyparietus*	*B. chuannanensis*	*B. feae*	*B. intermedia*	*B. wenshanensis*
SVL of males (in mm)	88.1–93.2 (n = 5)	76.8–82.7 (n = 8)	70.7–83.5 (n = 13)	70.0 (n = 1)	91.6–123.0 (n = 4)	88.5–113.0 (n = 6)	91.4–109.4 (n = 12)	78.5–94.9 (n = 5)	86.0–103.0 (n = 7)	83.8–85.1 (n = 2)
SVL of females (in mm)	108.7–111.2 (n = 2)	88.6 (n = 1)	86.2 (n = 1)	80.1–84.9 (n = 3)	124.0–168.0 (n = 3)	118.5–131.0 (n = 3)	–	–	92.0 (n = 1)	–
Tongue	Shallow notched behind	Feebly notched behind	Deeply notched behind	Feebly notched behind	Feebly notched behind	Feebly notched behind	Feebly notched behind	Feebly notched behind	Feebly notched behind	Feebly notched behind
Dermal ridge or glandular fold on dorsum	Absent	Absent	Absent	Absent	Dermal ridge on dorsum	Absent	Absent	Absent	Glandular fold on dorsum	Absent
Small, conical, horn-like tubercles	Absent	Absent	Absent	Absent	Absent	Present on pectoral region, lateral belly to lower flank of body, ventral surface, and rear of limbs	Absent	Absent	Absent	Absent
Webbing formula of male	I (1½)-(2) II (1½)-(3) III (2½)-(4) IV (4)-(2) V	I (1½)-(2) II (1½)-(3) III (2½)-(4) IV (4)-(2) V	I (1½)-(2) II (1½)-(3) III (2½)-(3⅔) IV (3⅔)-(2) V	I (1)-(2+) II (2-)-(3-) III (2½)-(4-) IV (4++)-(2-) V	–	I (1½)-(2+) II (1½)-(3) III (2⅓)-(3⅔) IV (3⅔)-(2-) V	I (1½)-(2++) II (2)-(3++) III (3)-(4) IV (4++)-(2½) V	I (2)-(2++) II (2-)-(3) III (2⅔)-(4) IV (4)- (2⅔) V	–	I (2-)-(2++) II (2-)-(3++) III (2½)-(4) IV (4+)-(2⅔) V
Webbing formula of female	I (2)-(2+) II (1⅔)-(3) III (3-)-(4) IV (4+)-(2½) V	I (2)-(2+) II (1⅔)-(3) III (3-)-(4) IV (4+)-(2½) V	I (1½)-(2+) II (1½)-(3) III (2½)-(4-) IV (4-)-(2) V	I (2½)-(2+) II (2-)-(3+) III (2½)-(4) IV (4++)-(2) V	–	I (1½)-(2+) II (1½)-(3) III (2⅔)-(4-) IV (4-)-(2⅔) V	–	–	–	–
Lateral fringes on toes of male	One third as broad as distal toe phalanx	One third as broad as distal toe phalanx	One fourth as broad as distal toe phalanx	Narrow	–	Half as broad as distal toe phalanx	One fifth as broad as distal toe phalanx in CIB 98A0045	One fourth as broad as distal toe phalanx	–	Narrow
Position of tibiotarsal articulation reaching	Commissure of jaw	Commissure of jaw	Commissure of jaw	Angle of mouth	Axilla in females, angle of mouth in males	Commissure of jaw	Shoulder or commissure of jaw	Shoulder in females, commissure of jaw in males	Commissure of jaw	Angle of mouth
Transversal stripe on chest in tadpole	A transversal stripe on chest	Absent	A transversal stripe on chest	–	Dark reticulation on belly	–	A transversal stripe on chest	Six dark bands on belly and a large rapezoid on chin	–	–

–: Not available

Figure 9. Egg protection behavior in *Brachytarsophrys wui* sp. nov. **A.** Eggs of *B. wui*; **B.** Under rock, adult male on right red mark with eggs on left. Photos by Jing-Cai Lyu.

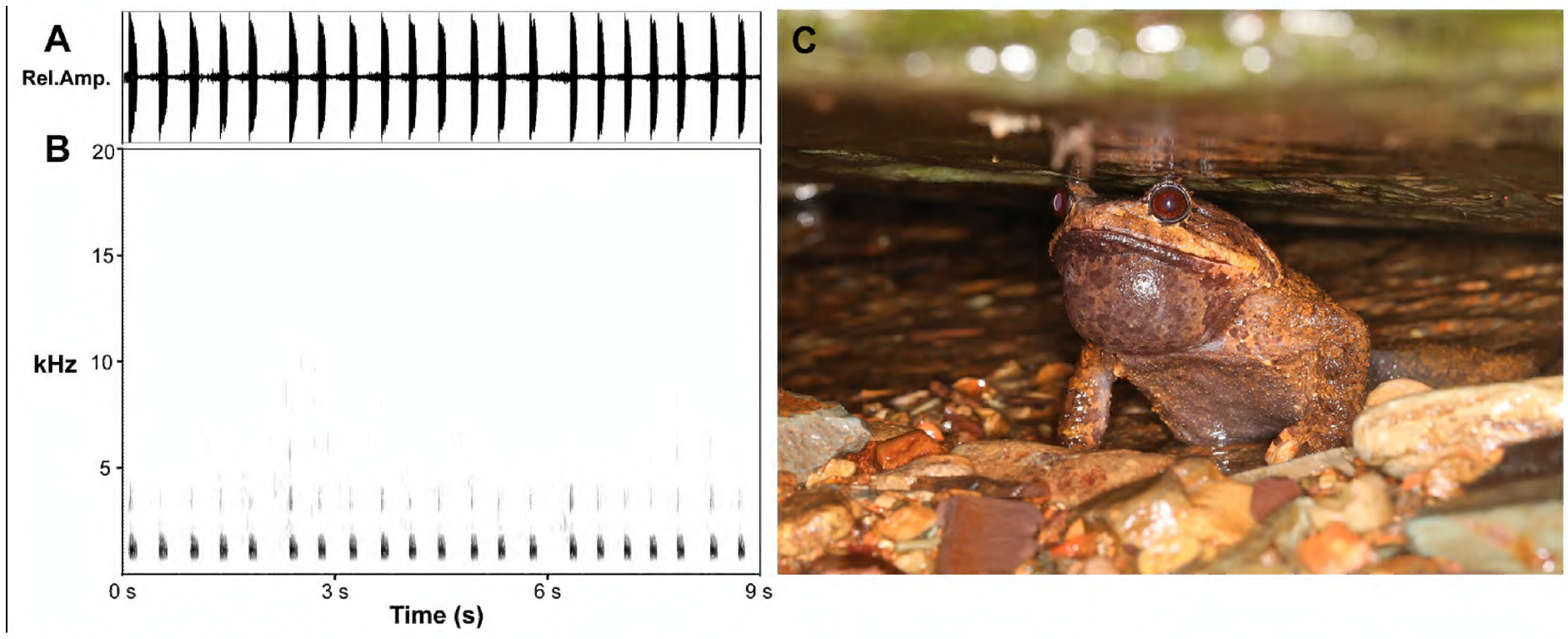

Figure 10. The advertisement call of the holotype *Brachytarsophrys wui* sp. nov. Expanded ~ 9 s waveform with Oscillogram top. and corresponding audio spectrogram bottom (**A, B**). Photos by Jing-Cai Lyu (**C**).

and rear of limbs (vs. presence). *Brachytarsophrys wui* sp. nov. differs from *B. feae* (Boulenger, 1886) by having slightly larger webbing of relative length I (1½)-(2) II (1½)-(3) III (2½)-(4) IV (4)-(2) V in males (vs. toes with rudimentary webbing, narrow, less than one fourth of distal toe phalanx in males), lateral fringes on toes of males one third as broad as distal toe phalanx (vs. one fourth as broad as distal toe phalanx), a single transverse stripe on chest in tadpole (vs. several transversal stripes on chest in tadpole), and the absence of stellate bony deposits on each side of parietal region (vs. presence). *Brachytarsophrys wui* sp. nov. differs from *B. qiannanensis* (Li et al. 2022) by having larger body size (vs. SVL 80.1–84.9 mm in female, 70.1 mm in males); head width about 1.5 times head length (vs. head width about 1.7 times head length); the male specimen with indistinct brown nuptial pad present on the dorsal surface of the first and second fingers base (vs. a brown nuptial pad present on the dorsal surface of the first finger). *Brachytarsophrys wui* sp. nov. differs from *B. wenshanensis* (He et al. 2024) by having larger body size (vs. SVL 83.8–85.1 mm in males); head width about 1.5 times head length (vs. head width about 1.2 times head length); the male specimen with indistinct brown nuptial pad present on the dorsal surface of the first and second fingers base (vs. lacking nuptial pad in male).

Discussion

In identifying a previously undocumented lineage, our study further reveals that species diversity within the genus *Brachytarsophrys* remains insufficiently explored. Recent phylogenetic analyses have revealed multiple genetic lineages of *Brachytarsophrys* that may represent new species (Li et al. 2020; Li et al. 2022; He et al. 2024). Our results bring the total number of species of *Brachytarsophrys* to ten; they are distributed in tropical and subtropical eastern and southeastern mainland Asia, including southern China, Myanmar, Vietnam, Laos, and northern Thailand. Two species, *B. carinense* and *B. intermedia*, occur in the Indochina Peninsula, eight of *Brachytarsophrys* known from China distributed in Southern China (Li et al. 2020; Li et al. 2022; He et al. 2024; Amphibian

China 2025). *B. feae* is currently recognized from northern Myanmar and Yunnan Province (Boulenger 1886); *B. chuannanensis* is distributed in Hejiang and Junlian counties, Sichuan Province (Fei and Ye 2001); *B. popei* populations occur in Taoyuangdong Nature Reserve, Hunan Province, adjacent Mt. Jinggang, Jiangxi Province, and Nanling Reserve, Guangdong Province (Zhao et al. 2014); *B. orientalis* is only known from the Jiulianshan Nature Reserve in Jiangxi Province and Gutian Township and Huboliao Nature Reserve in Fujian Province (Li et al. 2020); *B. platyparietus* is recognized from Duodihe of Dayao County, Mt. Mopan of Xinping County, Yilong Township of Shiping County, Mt. Jinzhong of Longlin County, Mt. Fanjing of Tongren City, Yumen Township of Yanbian County, indicating its potential distribution areas; *B. qiannanensis* is known from the type locality, Libo County, Guizhou Province (Li et al. 2022); *B. wenshanensis* is currently known from Xiaoqiaogou District, Xichou County, Wenshan National Nature Reserve, Wenshan Autonomous Prefecture, Yunnan Province (He et al. 2024).

Guizhou province warrants particular attention in this effort. It holds a rich biodiversity in its karst habitats and is a high priority for conservation (Jiang and Ryosuke 2009). So far, the genus *Brachytarsophrys* of *B. carinense*, *B. chuannanensis* and *B. qiannanensis* were recorded from Leishan, Anlong, Jiangkou and Libo Counties of Guizhou Province (e.g., Wu et al. 1987; Fei et al. 2009; Lyu et al. 2014; Li et al. 2022). Based on molecular phylogenetic analyses and morphological comparisons, Li et al. (2020) suggested the population of *B. carinense* from Jiangkou County should be reidentified as *B. platyparietus*, the population of *Brachytarsophrys* from Anlong County may be *B. platyparietus* and that from Leishan County may be *B. popei* or *B. orientalis*. However, the taxonomic assignments of the specimens are still doubtful because Li et al. (2020) did not provide more morphological and bioacoustics information of this specimen for comparisons with other species.

Brachytarsophrys wui sp. nov. had not been documented in previous studies, demonstrating that with intensive, targeted surveying there may be more undocumented species to be found (Wu et al. 1987; Li et al. 2020; Li et al. 2022). Future intensified surveys combined with the incorporation of molecular and acoustic data, especially in previously unexplored areas, will likely continue uncovering new species of *Brachytarsophrys*.

We had conducted many investigations in all areas of the Mt. Foding Nature Reserve from 2018 to 2020, and some individuals were found in a narrow distribution in Mt. Foding. Based on field investigation, it is shown that *B. wui* sp. nov. has a narrow distribution in Mt. Fodingn National Nature Reserve, being confined exclusively to Lengshui River and Bingjia River. Additionally, this species exhibits high habitat specificity, its breeding habitat is characterized by relatively fast-flowing water, with riverbeds containing large cobblestones (Fig. 11). The distribution locality underwent intensive anthropogenic disturbance via deforestation in the 1980s followed by further disturbance from the development of modern tourism. The narrow distribution of this species increases its vulnerability to extinction from habitat loss and degradation. Greater protections for this habitat are warranted in order to safeguard this and other endemic species.

Figure 11. Breeding habitat at the type locality of *Brachytarsophrys wui* sp. nov. in Mt. Foding National Nature Reserve, Shiqian County, Guizhou province, China.

Acknowledgements

This study was supported by Mt. Foding National Nature Reserve Bureau of Shiqian County, Guizhou Province; The Diversity of Amphibians and Reptiles in Mt. Foding National Nature Program (2018, 2019), Forestry and Grassland Ecological Protection and Restoration Fund of Guizhou Province (2025). We thank Yang Fang, Yuanhong Li, Hongjiang Li and Xingjun Hu from Mt. Foding National Nature Reserve Bureau for their help with fieldwork as well as Zhiyong Yuan and Yanhong He from Southwest Forestry University for help with laboratory work. We greatly thank Jinmin Chen, Hollis A. Dahn for checking and revising the grammar of the article.

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Supplementary material 1

Genetic distances between members of *Brachytarsophrys* in 16S rRNA sequences

Authors: Jing-Cai Lyu, Sheng-Lun Zhou, Ting-Bi Zhang, Yang Fang, Si-Yu Ran, Liang-Liang Dai, Xuan-Kong Jiang, Han-Wu Long, Guo Yuan

Data type: xlsx

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Link: https://doi.org/10.3897/zse.102.161437.suppl1

Supplementary material 2

Genetic distances between members of *Brachytarsophrys* in COI sequences

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Data type: xlsx

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